

Charophyte Communities in the Ein Afeq Natural Reserve, Israel

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Abstract First study of new locality in the Ein Afeq Natural Reserve with charophytes in the Akko Plain of Northern Israel has been implemented for revealing of algal diversity and ecological assessment of the water object environment by bio-indication methods. Altogether seventy one species of algae including two of them macro-algae *Chara vulgaris* Linnaeus and *C. connivens* P. Salzmänn ex A. Braun were revealed. *Chara* was found in massive growth in two newest pools. Bio-indication and chemical variables characterize the pools environment as eutrophic, low- to middle organic polluted, Class II-III of water quality. Water was fresh, temperate, low alkaline, and well saturated by oxygen. Succession of algal community and higher aquatic plants shows increasing of organic pollution from 2005 to 2015 as a result of recreation impact. We can recommend the Ein Afeq pools for monitoring of natural aquatic object in the Akko Plane as reference site, and *Chara vulgaris* and *C. connivens* as climatic and successional indicators.

Keywords Charophytes, Ecology, Bio-indication, Ein Afeq Natural Reserve, Israel

words meteoric in appearance. Several species mostly largest ones are perennial and may form communities which are the most stable in clear deep stratifying lakes [1].

The charophytes prefer alkaline water environment which forms on the carbonates that are very distributed in studied region. During last year's we find new, unstudied aquatic objects in which were identified charophyte algae [2]. They are apparently absent in single Israeli large lake (Lake Kinneret) for a long time as might be concluded from published data and our observations. Nearby regions, such as Turkey, also give us charophyte algae new localities that we studied in respect of species diversity and bio-indication of its environment [3,4]. Especially important to revealed the charophytes diversity in the Natural Reserves with saving diversity programs.

The aim of present study is to identify of charophyte and microscopic algal diversity that studied firstly in the Ein Afeq Natural Reserve pools, and to assess the pools environment by bio-indication methods on the base of revealed algae and water chemistry.

1. Introduction

Diversity of the charophytes (Charales, Streptophyta) in the Eastern Mediterranean is studied in initial stage. But these macroscopic autotrophic algae may be very important components of vegetation in several types of water bodies and may be used as a bio-indicator of ecosystem state, water quality, and ecosystem recovery and reservoir management efficiency.

Charophytes easily colonize habitats in new water bodies as well as ones formed as result of disturbance. They are well-known as pioneer plants i.e. key species in first stages of succession subsequently replaced by angiosperms or filamentous algae especially as a result of eutrophication. In temporal water bodies charophytes are ephemeral or in other

2. Materials and Methods

2.1. Sampling and Laboratory Studies

We collected fifteen living and fifteen fixed algological samples, twenty samples of charophytes and fifteen samples of water during six field trips from 2005 to 2015 in the Ein Afeq Natural Reserve pools (Figure 1).

Algological samples were collected by scratching and scooping, placed in 15 ml plastic tubes, and partly fixed with 3% neutral formaldehyde solution, as well as partly not fixed and transported to the laboratory in the ice box.

We also studied eight dry samples of charophytes that were collected by Prof Yaakov Lipkin in February 1971 and April 1972 in the Ein Afeq area but without relation of individual waterbody. These samples were given to us for study under the grant of Israeli Taxonomical Initiative and are placed in the Tel Aviv University collection.



Figure 1. The Ein Afeq Natural Reserve in the Akko Plain of Northern Israel, marked with black rectangle

Charophytes were treated with 2-3% HCl to remove calcium carbonate. After washing several times with distilled water the material was studied with Nikon stereomicroscope with distilled water the material was air-dried on cover glasses and mounted in Naphrax®. The structure elements were observed with a Carl Zeiss Stereo Discovery V12 stereomicroscope equipped with an AxioCam MRs-5 digital camera and Axiovision 4.8 software, and DinoLight camera, the diatoms were observed with Nikon Eclipse Ci and SWIFT light microscopes (LM) and photographed with Leica 520 DC in the Institute of Evolution, University of Haifa, and the Central Siberian Botanical Garden with help of international handbooks [5,6]. Charophyte and microscopic algae abundance were assessed as abundance scores according 6-score scale [7].



Figure 2. The Ein Afeq Natural Reserve, Pools with charophytes are numbered, central antique building of ancient mill marked with white arrow, the water Well pointed by white arrowhead

Algae and cyanobacteria were studied with the SWIFT and OLYMPUS dissecting microscopes under magnifications 740x–1850x from three repetitions of each sample and were photographed with a DC (OMAX A35100U). The diatoms were prepared by the peroxide technique [8] modified for glass slides [9] and were placed in the Naphrax® resin from two repetitions of each sample.

Temperature, Electrical conductivity (EC), Acidity (pH), and TDS were measured with HANNA HI 9813-0. The concentration of N-NO₃ was measured with HANNA HI 93728.

Index saprobity *s* was calculated according to [10]. Index of aquatic ecosystem sustainable was calculated according to [7,11] as (1):

$$\text{WESI} = \text{Rank } S / \text{Rank } \text{N-NO}_3, \quad (1)$$

Where: Rank *S* – rank of water quality on the Sladeček's indices of saprobity; Rank N-NO₃ – rank of water quality on the nitric-nitrogen concentration (Table 1).

If WESI is equal to or larger than 1, the photosynthetic level is positively correlated with the level of nitrate concentration. If the WESI is less than 1, the photosynthesis is suppressed presumably according to toxic disturbance [7].

2.2. Description of the Study Site

The Ein Afeq Nature Reserve, on the outskirts of Kiryat Bialik, Northern Israel, is the last vestige of the Nahal Na'aman swamps, which once extended all the way to the Acre valley (Figure 1).

The Ein Afeq Spring is in the nature reserve. A wooden path over the swamp passes near typical riverbank flora, such as tamarisk trees, blackberry hedges, reeds, and bulrushes. Plants growing in the water include duckweed and pond weeds [12]. When the water level rises in the winter, birds flock to the reserve: pelicans, cranes, pygmy cormorants, gray herons, moorhens, European coots, and black-winged stilts, to name just a few. In the winter and spring, the reserve is carpeted with flowers. A fine spot for admiring the blossoms is Tel Afeq, situated on a low sandstone hill on the edge of the reserve. On the northern slope of Tel Afeq is a two-story fortified building from the Crusader period [12].

System of natural and artificial pools is placed in the reserve territory (Figures 2-9) on altitude about 8 m above sea level with coordinates 32°50' N, 35°06' E (Table 1). They are small, about 3-120 m in diameter and up to one m deep, filled by natural ground waters year-round, and used for education and recreation. Water source of the Na'aman River start from the Pool 1 and Well from the right side and follow to Pools 2 and 3 from the left side. The pools 4 and 5 are newly artificial. Each pool in the reserve is under management in purpose to save diversity of plants and animals.

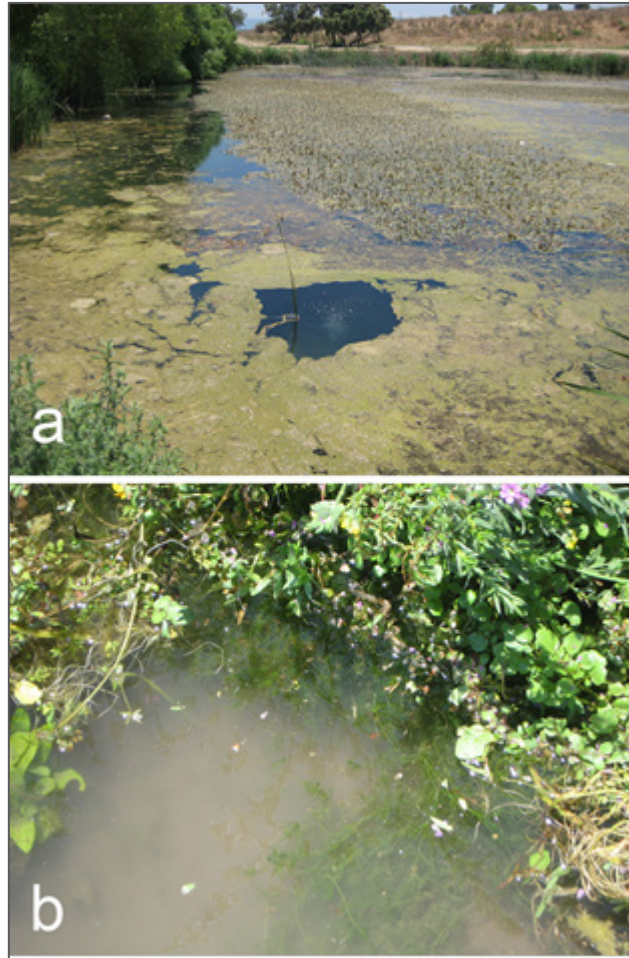


Figure 3. Pool 2: a – water pipe bubbles can be seen in the central part, July 2011; b – *Chara vulgaris* L. population near the pool bank in July 2011.



Figure 4. Pool 1 development from the same point of view: a – July 2011, c – March 2012, d – March 2015; b – *Chara vulgaris* initial population in July 2011

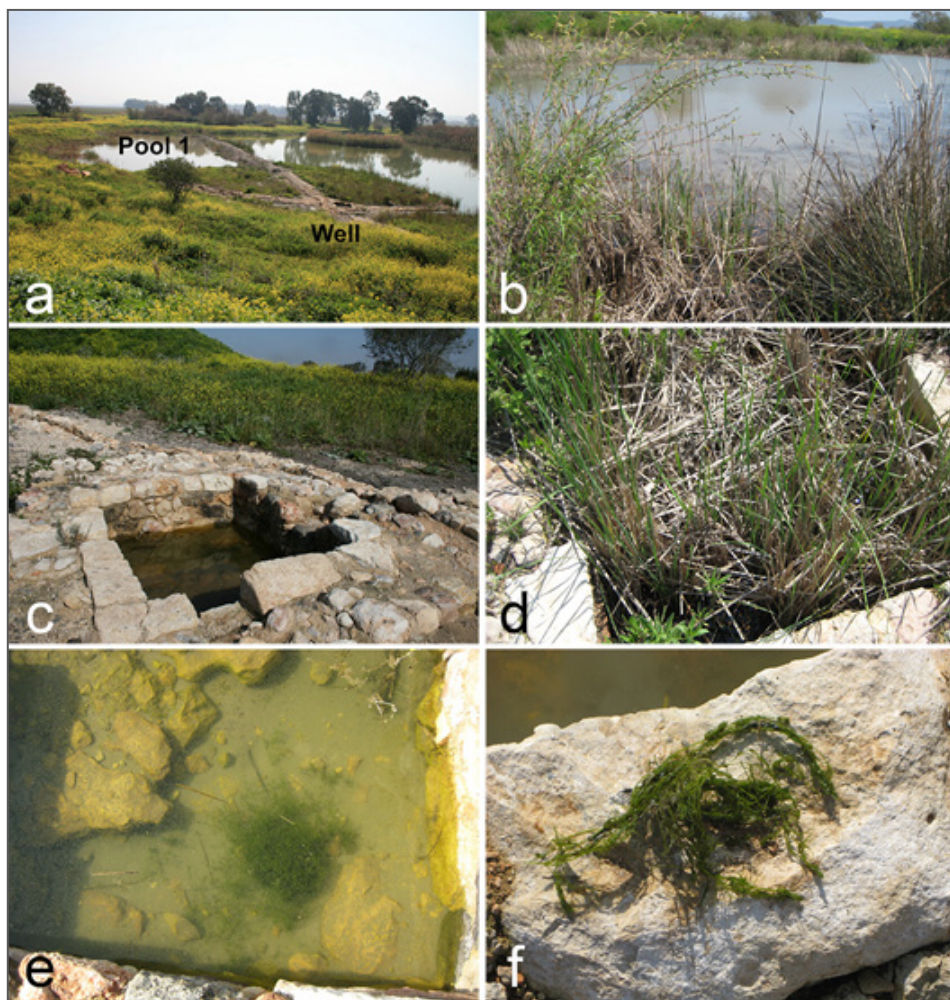


Figure 5. Pool 1 and Well development: a – March 2012 with water and nuded banks; b – Pool 1 in March 2015 with water and plants on the banks; c – Well in March 2012 with water and charophytes; d – Well in March 2015 without *Chara* and water; e, f – *Chara vulgaris* in Well, March 2012.

Table 1. Averaged chemical and biological variables of the Ein Afeq pools with charophytes in 2011-2015

Pool	North	East	Season	Year	Conductivity, mS cm ⁻¹	N-NO ₃ , mg l ⁻¹	pH	TDS, mg l ⁻¹	T, °C	No. of Species	Index S	Index WESI
1	32°50'48.0''	35°06'58.0''	Summer	2011	3.09	0.60	8.0	-	33.0	25	1.74	1.0
Well	32°50'48.0''	35°06'56.0''	Winter	2012	2.76	0.70	7.1	1733	15.7	10	1.84	1.0
2	32°50'76.9''	35°06'83.8''	Summer	2011	2.44	7.40	6.9	1814	25.6	26	1.71	0.4
3	32°50'84.4''	35°06'76.7''	Summer	2011	2.54	2.70	7.4	1902	28.5	5	1.53	0.5
4	32°50'53.0''	35°06'43.0''	Winter	2012- 2015	0.39	0.87	8.5	282.3	16.8	37	1.58	1.0
5	32°50'50.2''	35°06'44.5''	Winter	2015	1.32	1.00	9.4	955.6	20.0	14	1.43	0.8

Table 2. Algal diversity with abundance scores and species ecological preferences (according to [7,13]) in the Ein Afeq Natural Reserve pools in 2005-2015

Taxa	P1	P2	P3	P4	P5	Well	Hab	T	Reo	pH	Sal	Sap	D	Het	Tro
Cyanobacteria															
<i>Chamaesiphon polonicus</i> (Rostafinski) Hansgirg	0	0	0	0	6	0	B	-	aer	-	-	o-x	-	-	o
<i>Chroococcus turgidus</i> (Kützing) Nägeli	0	2	0	0	0	1	P-B	-	aer	alf	hl	o	-	-	-
<i>Heteroleibleinia kuetzingii</i> (Schmidle) Compère	0	0	0	0	1	0	B	-	st-str	-	-	o-b	-	-	-
<i>Lyngbya major</i> Meneghini ex Gomont	0	0	0	4	1	0	B	-	-	-	-	b	-	-	m
<i>Lyngbya</i> sp.	4	0	0	0	0	0	-	-	-	-	-	-	-	-	-
<i>Merismopedia punctata</i> Meyen	3-4	3	0	0	0	0	P-B	-	-	ind	i	o-a	-	-	me
<i>Merismopedia tenuissima</i> Lemmermann	0	0	0	0	0	6	P-B	-	-	-	hl	b-a	-	-	e
<i>Microcoleus amoenus</i> (Gomont) Struncky, Komárek & J.R.Johansen	2-3	0	0	0	0	0	P-B	-	st-str	-	-	x	-	-	-
<i>Oscillatoria limosa</i> C.Agardh ex Gomont	0	0	0	4	0	0	P-B	-	st-str	-	hl	b	-	-	e
<i>Oscillatoria</i> sp.	1	0	0	0	0	0	-	-	-	-	-	-	-	-	-
<i>Pseudanabaena redeckeii</i> (Goor) B.A.Whitton	0	2	0	0	0	0	P-B	sera	-	-	-	b-o	-	-	me
<i>Spirulina major</i> Kützing ex Gomont	1-4	0	0	0	0	0	P-B	-	st	-	hl	a	-	-	-
Euglenozoa															
<i>Euglena deses</i> Ehrenberg	2	0	0	0	0	0	P-B	-	st-str	ind	mh	m	-	-	-
<i>Euglena texta</i> (Dujardin) Hübner	0	0	0	1	0	0	P	eterm	st-str	ind	-	b	-	-	-
<i>Lepocinclis acus</i> (O.F.Müller) Marin & Melkonian	2	0	0	2	0	0	P	eterm	st	ind	i	b	-	-	-
<i>Phacus limnophilus</i> (Lemmermann) E.W.Linton & A.Karnkowska-Ishikawa	3	0	0	0	0	0	P-B	eterm	st-str	-	-	o-b	-	-	-
Ochrophyta															
<i>Tribonema viride</i> Pascher	1	0	0	0	0	0	P-B	-	-	-	i	o	-	-	-
Bacillariophyta															
<i>Achnanthydium minutissimum</i> (Kützing) Czarnecki	0	1-5	0	2	0	6	B	eterm	st-str	alf	i	b	es	ate	o-e
<i>Amphora ovalis</i> (Kützing) Kützing	0	2	1	0	0	0	B	temp	st-str	alf	i	a-b	sx	ate	e
<i>Amphora pediculus</i> (Kützing) Grunow ex A.Schmidt	0	0	0	0	6	0	B	temp	st	alf	i	o-a	sx	ate	e
<i>Cocconeis placentula</i> Ehrenberg	0	3-5	3	3	0	0	P-B	temp	st-str	alf	i	o-b	es	ate	e
<i>Craticula cuspidata</i> (Kützing) D.G.Mann	1	0	0	1	0	0	B	temp	st-str	alf	i	o	es	ate	e
<i>Craticula halophila</i> (Grunow) D.G.Mann	0	1	0	0	0	0	B	-	st-str	alf	mh	a	es	ate	e
<i>Cylindrotheca closterium</i> (Ehrenberg) Reimann & J.C.Lewin	0	0	0	1	0	0	B	-	-	-	mh	-	-	-	-
<i>Cymbella affinis</i> Kützing	1	2	0	0	0	0	B	temp	st-str	alf	i	b-o	sx	ats	e

<i>Cymbella turgidula</i> Grunow	3	3	0	1	0	0	B	-	st-str	ind	-	-	es	-	-
<i>Epithemia adnata</i> (Kützing) Brébisson	0	0	0	4	6	0	B	temp	st	alb	i	b-a	sx	ats	me
<i>Fragilaria radians</i> (Kützing) D.M.Williams & Round	0	0	0	0	0	2	B	-	-	alf	i	o	sx	-	-
<i>Gomphonema gracile</i> Ehrenberg	0	0	0	0	3	0	P-B	temp	st	alf	i	b-o	es	ats	m
<i>Gomphonema longiceps</i> Ehrenberg	0	2	0	0	0	0	B	-	str	ind	i	o-b	es	-	-
<i>Gomphonema parvulum</i> (Kützing) Kützing	1	1	0	1	1	1	B	temp	str	ind	i	x	es	hne	e
<i>Gomphonemopsis obscura</i> (Krasske) Lange-Bertalot	0	0	1	0	0	0	B	-	-	-	hl	-	-	-	-
<i>Grunowia sinuata</i> (Thwaites) Rabenhorst	0	6	0	0	0	0	B	-	str	ind	i	b-a	-	ats	m
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	4	0	0	3	0	0	B	cool	st-str	alf	i	o-x	-	ate	e
<i>Halamphora holsatica</i> (Hustedt) Levkov	2	0	0	0	0	0	P	-	st-str	-	hl	-	-	-	-
<i>Haslea spicula</i> (Hickie) L.Bukhtiyarova	2	0	0	0	0	3	P-B	-	-	-	mh	-	-	-	-
<i>Navicula cincta</i> (Ehrenberg) Ralfs	0	0	0	1	0	0	B	warm	st-str	alf	hl	x-o	es	ate	e
<i>Navicula exigua</i> Gregory	3	4	0	1-3	0	2	B	-	str	alf	i	x-o	es	ats	e
<i>Navicula recens</i> (Lange-Bertalot) Lange-Bertalot	2	0	0	1	0	0	P-B	-	-	alf	i	o-b	es	-	e
<i>Navicula rhynchocephala</i> Kützing	2	2	0	0	0	0	B	-	-	alf	hl,i	b	-	ate	o-e
<i>Nitzschia commutata</i> Grunow	0	0	0	1	1	0	B	-	-	-	mh	-	-	-	-
<i>Nitzschia fonticola</i> (Grunow) Grunow	1-6	3	1	2	0	0	B	-	st-str	alf	oh	o-b	-	ate	me
<i>Nitzschia gracilis</i> Hantzsch	0	0	0	2	0	0	P-B	temp	st-str	ind	i	o-x	sp	-	m
<i>Nitzschia palea</i> (Kützing) W.Smith	2	2	0	2	0	0	P-B	temp	-	ind	i	o-x	sp	hce	he
<i>Nitzschia solita</i> Hustedt	1	0	0	1	0	2	B	-	st	alf	mh	a-b	es	-	e
<i>Nitzschia tryblionella</i> Hantzsch	0	0	0	2	0	1	B	-	st-str	alf	hl,mh	o	-	ate	e
<i>Nitzschia vermicularis</i> (Kützing) Hantzsch	0	0	0	2	0	0	B	-	str	alf	i	o	-	-	o-e
<i>Rhopalodia gibba</i> (Ehrenberg) Otto Müller	0	0	0	1	0	0	B	temp	-	alb	i	x-o	es	-	-
<i>Sellaphora pupula</i> (Kützing) Mereschkovsky	1	0	0	0	0	0	B	eterm	st	ind	hl	x-o	sx	ate	me
<i>Stephanodiscus hantzschii</i> Grunow	0	2	0	0	0	0	P	temp	st	alf	i	a-b	es	hne	he
<i>Surirella ovalis</i> Brébisson	0	1	0	0	0	0	B	-	st-str	ind	i	o-a	es	-	-
<i>Tropidoneis gibberula</i> (Grunow) Cleve	0	0	0	4	0	0	B	-	-	-	-	-	-	-	-
<i>Tryblionella angustata</i> W.Smith	0	0	0	1	0	0	P	-	st	alf	i	x-b	sx	ats	m
<i>Tryblionella hungarica</i> (Grunow) Frenguelli	0	0	0	1	0	0	P-B	-	-	alf	mh	a-b	sp	ate	e
<i>Tryblionella kuetzingii</i> Álvarez-Blanco & S.Blanco	0	1	0	0	0	0	B	-	st-str	alf	mh	b	es	ate	e
<i>Ulnaria danica</i> (Kützing) Compère & Bukhtiyarova	0	1	0	0	0	0	P-B	temp	-	alf	i	x-b	es	-	-
<i>Ulnaria ulna</i> (Nitzsch) P.Compère	0	1-4	0	1	0	0	B	temp	st-str	alf	i	b-o	es	ate	o-e

Chlorophyta															
<i>Cladophora glomerata</i> (Linnaeus) Kützing	0	0	0	6	6	0	P-B	-	st-str	alf	i	b-o	-	-	-
<i>Desmodesmus brasiliensis</i> (Bohlin) E.Hegewald	0	0	0	2	0	0	P-B	-	st-str	-	-	b	-	-	-
<i>Eudorina elegans</i> Ehrenberg	0	0	0	1	0	0	P	-	st-str	-	i	b	-	-	-
<i>Oedogonium</i> sp. ster.	1	0	0	3-6	5	0	-	-	-	-	-	-	-	-	-
<i>Scenedesmus apiculatus</i> var. <i>indicus</i> (Hortobágyi) Hortobágyi	0	3	0	0	0	0	P-B	-	st	-	-	-	-	-	-
<i>Scenedesmus obliquus</i> (Turpin) Kützing	0	4	0	0	0	0	P-B	-	st-str	-	i	b	-	-	-
Charophyta															
<i>Chara connivens</i> P.Salzmann ex A.Braun	0	0	0	4	3	0	B	-	-	-	-	-	-	-	-
<i>Chara vulgaris</i> Linnaeus	1	2	4	2-6	0	2	B	-	st-str	-	-	o	-	-	-
<i>Closterium acerosum</i> Ehrenberg ex Ralfs	0	0	0	3	0	0	P-B	-	st-str	ind	i	a-b	-	-	me
<i>Coleochaete pulvinata</i> A.Braun	0	0	0	0	6	0	Ep	-	st	-	-	o	-	-	-
<i>Cosmarium punctulatum</i> Brébisson	0	0	0	1	1	0	P-B	-	-	acf	hb	o	-	-	-
<i>Mougeotia</i> sp. ster.	0	4	0	0	0	0	B	-	-	-	-	o	-	-	-
<i>Spirogyra</i> sp. ster.	0	2	0	1-4	0	0	-	-	-	-	-	-	-	-	-
<i>Zygnema</i> sp. ster.	0	0	0	1	0	0	B	-	-	-	-	x-b	-	-	-

Note: Ecological types (Hab): Ep, epiphyte; B, benthic; P-B, planktonic-benthic; P, planktonic. Temperature (T): cool, cool water inhabitant; temp, temperate waters inhabitant; etern, eurythermic; warm, warm waters inhabitant; sera, H₂S indicator. Streaming and Oxygenation (Reo): aer, aerophile; str, streaming waters inhabitant; st-str, low streaming waters inhabitant; st, standing water inhabitant. Acidity (pH): acf, acidophil; ind, indifferent; alf, alkaliphil; alb, alkalibiont. Halobity (Sal): hb, halophobe; i, oligohalobious-indifferent; hl, oligohalobious-halophilous; mh, mesohalobe. Saprobity (D): sp, saprophil; es, eurysaprob; sx, saxoxen. Saprobity (Sap): o, oligosaprob; o-a, oligo-alpha-mesosaprob; o-x, oligo-xenosaprob; x-o, xeno-oligosarob; x-b, xeno-beta-mesosaprob; x, xenosaprob; o-b, oligo-beta-mesosaprob; b-o, beta-oligosaprob; b, beta-mesosaprob; b-a, beta-alpha-mesosaprob; a-b, alpha-beta-mesosaprob; a, alpha-mesosaprob; m, metasaprob. Nitrogen uptake metabolism (Aut-Het) [13]: ats, nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate, nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne, facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; hce, nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen. Trophic state (Tro) [13]: o, oligotrafentic; m, mesotraphentic; me, meso-eutraphentic; e, eutraphentic; he, hypereutraphentic; o-e, oligo- to eutraphentic (hypereutraphentic).

Climatic condition of the Ein Afeq area is warm and temperate with more rainfall in the winter than in the summer and mean annual rainfall about 531 mm. The driest month is June, with 0 mm of rain. With an average of 141 mm, the most precipitation falls in January. The temperature here averages 20.4 °C. August is the warmest month of the year with averages 27.3 °C. January has the lowest average temperature of the year about 13.1 °C [14]. Therefore, Afeq environment is very favorable for the development of photosynthetic plants and charophyte algae which are truly photosynthetic organisms.

3. Results and Discussion

3.1. Chemical Composition of the Pools Water

Chemical variables were measured in two seasons: summer and winter. Table 1 shows that environment variables are fluctuated over pools and seasons. Water conductivity and TDS have wide range in two newest pools 1 and 4, whereas old stabile pools 2 and 3 are in the same range. Pool 5 represent small artificial betony construction

filled by natural water and is clearer. Water pH is higher in the newest pools. Remarkable that Pool 2, deepest, has average in neutral water. As can be seen in Figure 5a, the water comes to the Pool 2 not only from the bottom but also from artificial source. Nitrogen concentration varied in natural range. Water temperature fluctuated in climatic norm with highest (33 °C) in the shallow Pool 1 in summer. Therefore, the pools water can be characterized as fresh, low alkaline, moderate temperature, and low polluted [7,11]. Index of saprobity S is fluctuated between 1.43 in the newest Pool 5 and 1.83 in the Well, and reflects Class II to III of Water Quality.

3.2. Diversity and Ecology of Algae

We revealed seventy one species of algae (Table 2) diversity of which is rather fluctuated between pools. Diatoms prevail in oldest but replaced by charophytes, greens and cyanobacteria in newest pools 1, 4, and 5.

Two macrophyte algae *Chara connivens* (Figures 10-12) and *C. vulgaris* (Figure 13) were found in different pools. Structural elements and thallus habitat for both species confirm that our samples are in the typical diagnosis frames.

It is also widely distributed species in the Mediterranean countries and some climatic similar regions [15] (algaebase.org). *Chara vulgaris* is simply separated from the other members of the genus *Chara* as we revealed by AFLP analysis [16]. It is two species that we found as distributed in Israel in historical and present time [2]. *Chara vulgaris* is a common and found most frequently in comparison with other species.

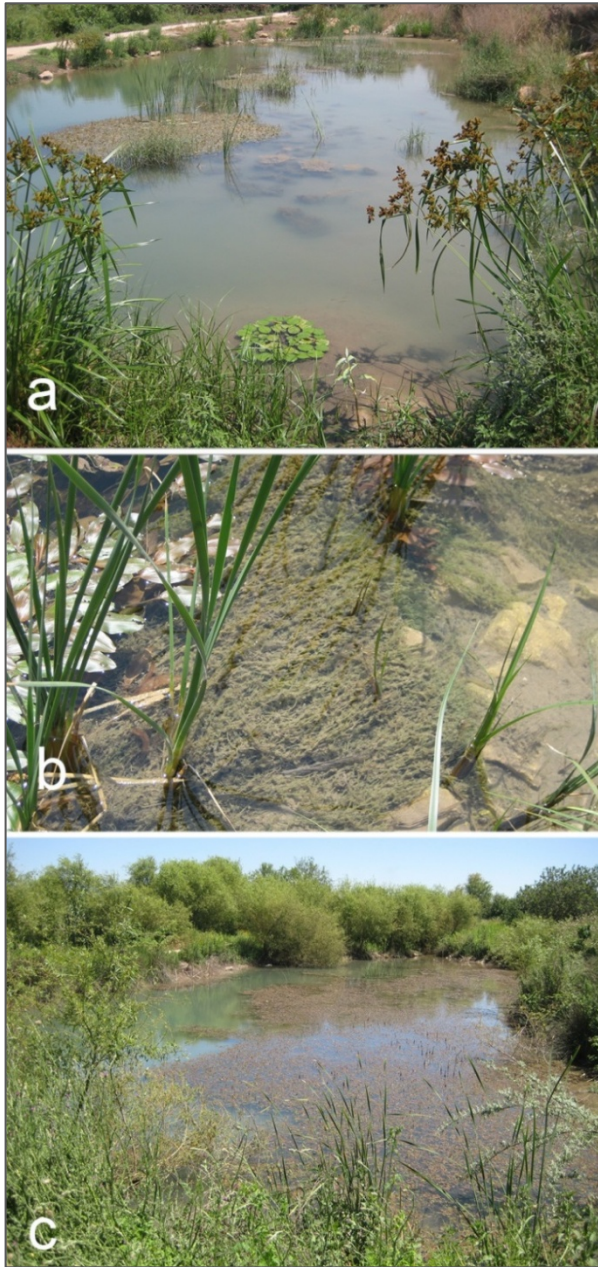


Figure 6. Pool 3: a – low covered by aquatic plants in July 2011; b – *Chara vulgaris*, in water input to the Pool 3, July 2011; c – Pool 3 covered by aquatic plants and without charophytes, March 2012.

Chara vulgaris was found in all studied pools except newest artificial Pool 5, but *C. connivens* was grow in pools 4 and 5 only. During ten year study of the Ein Afeq pools, we explore successional changes over the pools diversity. So, in 2005 the charophytes were absent. *Chara vulgaris* was found

in oldest Pool 2 in 2011 (Figure 3b) and Pool 3 (Figure 6b). Just created Pool 1 in 2011 contains *C. vulgaris* (Figure 4e), as well as Well in 2012 (Figure 5e,f) but only once during study period. Successional picture shows community in Pool 4 where in just created pool we found *C. vulgaris* in March 2012 (Figure 7b) that accompanied by *C. connivens* (Figure 7c) in initial stage and sterile. Later, in March and April 2015 the role of species in community change vice versa (Figure 8b,c).

Communities of *C. connivens* and *C. vulgaris* are pioneer communities mostly in ephemeral shallow or temporary water bodies, basic, rich in lime, sometimes sub-brackish, mesotrophic to slightly eutrophic for *C. connivens* and mesotrophic to eutrophic for *C. vulgaris* [17]. The last species is one of the most resistant to eutrophication among charophytes [6].

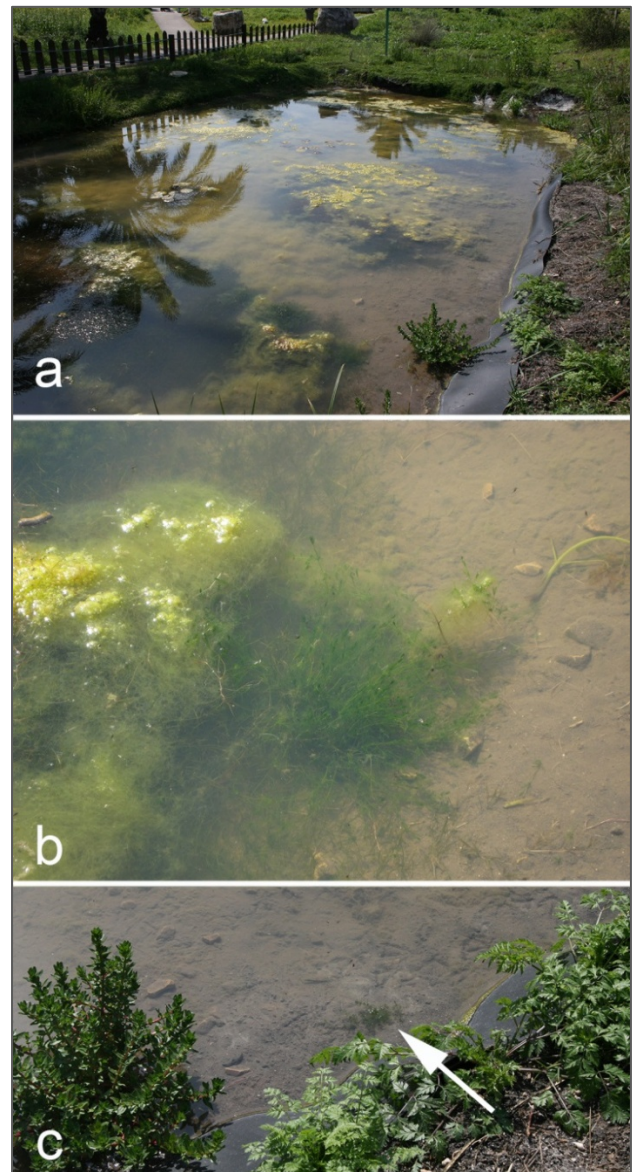


Figure 7. Pool 4: a – pool in initial stage in March 2012; b – *Chara vulgaris* massive grow with *Spirogyra* sp. ster., March 2012; c – initial thallus of *C. cf. connivens* Salzm. ex A. Braun ster. (white arrow), March 2012.

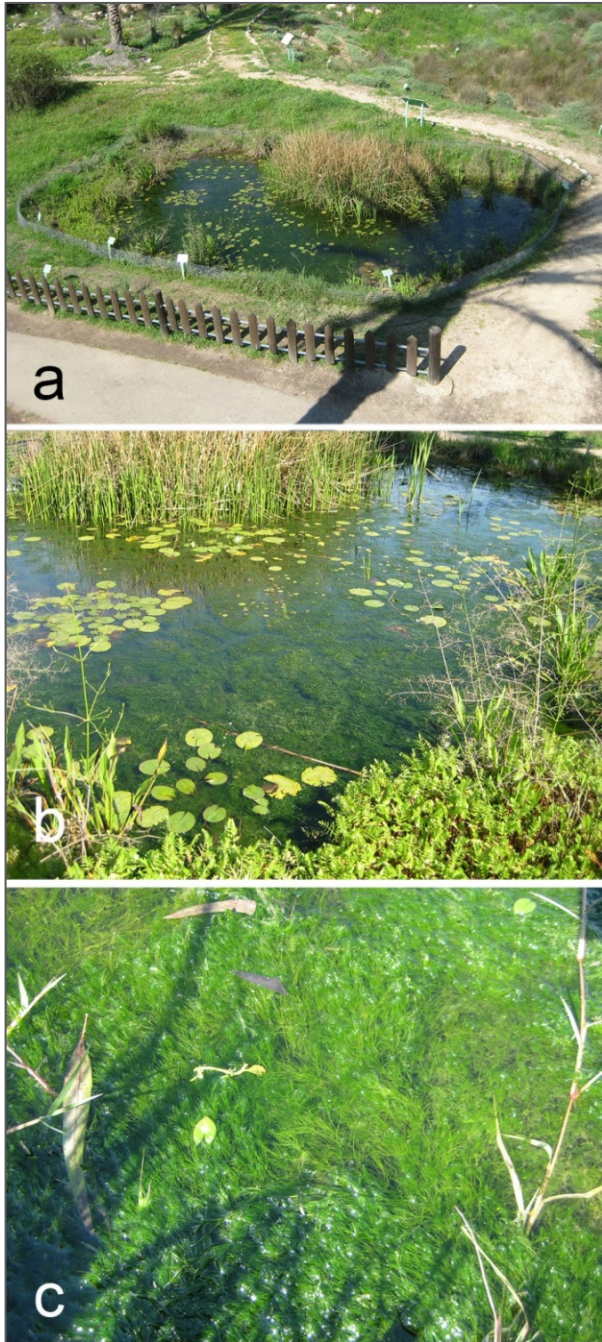


Figure 8. Pool 4: a – total view in 2015 with massive grows of charophytes and aquatic plants; b, c – *Chara cf. connivens* ster. massive grow, March 2015.

Community of each studied pool was different in species richness as well as in species contain. So, the richest communities were found in the newest Pool 4 with 14 species, and Pool 1 with 13 taxa. Deepest Pool 2 was with 12 taxa, and the betony new Pool 5 with 11. Algal communities in pools were reached by diatoms (Table 2). Pool 1 was dominated by *Nitzschia fonticola*, Pool 2 - by *Achnantheidium minutissimum* and *Cocconeis placentula*. Community in Pool 3 was mostly represented by *Chara vulgaris*. Pool 4 represents strongly domination of *Chara connivens* in its lather succession stage which was attached by mass of green

filaments of *Oedogonium* sp. ster. In Pool 5 community was new, *Chara connivens* thalluses were attached by the same *Oedogonium* filaments with diatom *Epithemia adnata*, green *Coleochaete pulvinata*, and cyanoprokaryote *Chamaesiphon* masses especially in the oldest *Chara* individuals. Algal community in the Well was pure and represent by *Chara vulgaris* with attached diatom *Achnantheidium minutissimum*.

We explore succession of higher water plants in studied pools as not only progressive, but even aggressive. So, the Well evolved from pure and low watered object to full overgrow by plants and empty of water and charophyte community (Figure 5c,d). Pool 1 also has progress in the aquatic plants during 2011-2015 (Figure 4a,c,d; 5b). In pool 3 we can see the same tendency in the Figure 6a,c. The charophytes left Pool 2, that can be related with increasing of organic pollution from 2005 to 2015 as a result of recreation which confirmed by nitric-nitrogen increasing up to 7.4 mg l⁻¹. Up to now we don't explore the advanced succession stages in the newest pools 4 and 5, but revealed tendency in incursion of high plants in oldest pools can be endangered other algal communities because high plants replacing charophytes. It helps us to assume that charophytes can be used as indicators of succession stage in aquatic communities. More of them, *Chara vulgaris* represents the exponential succession stage whereas *Chara connivens* replace it in succession advances.

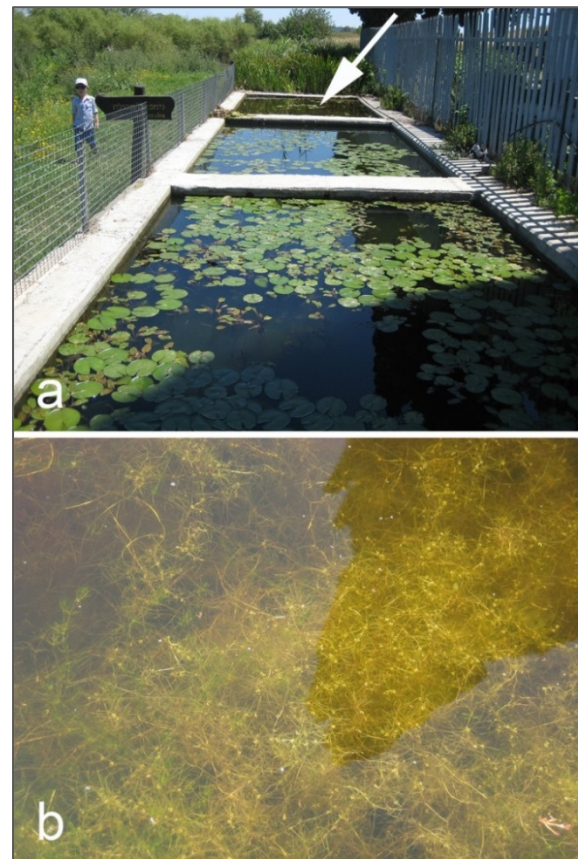


Figure 9. Pool 5: a – total view of betony cascade pools in 2015 with the last one as Pool 5, pointed by white arrow; b – *Chara connivens* grow, April 2015.



Figure 10. *Chara* cf. *connivens* ster. total view of thallus in Pool 4, March 2012.

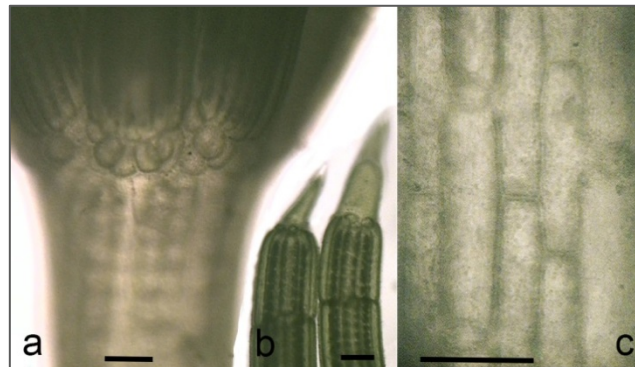


Figure 11. *Chara* cf. *connivens* ster. in Pool 4, March 2012: a – axis with stipulodes and base of whorl; b – branches end cells; c – axis with triplostichous axial cortex. Scale bars: a – 100 μ m; b – 50 μ m; c – 100 μ m.

In this case it is remarkable that charophytes in the Ein Afeq pools can not only change its diversity over the time but also survive with helps of oospores in the pools sediments. We don't find yet other species of charophytes in the Ein Afeq nevertheless its pools and climatic conditions represent well environment for its surviving.



Figure 12. *Chara connivens* in Pool 5, April 2015: a – axis with whorl of branchlets and oogonia; b – oogonium; c – axis with stipulodes, base of whorl. Scale bars: 200 μ m.

We are grateful to have access to the Tel Aviv University herbarium in the frame of Israeli Taxonomic Initiative Grant. Dr. Lipkin collection let us to assume that we don't exhaustive the charophytes list in this protected area and in future can find also *Chara vulgaris* var. *longibracteata* (Kütz.) J. Gr. et B. Webst. (Figure 14).

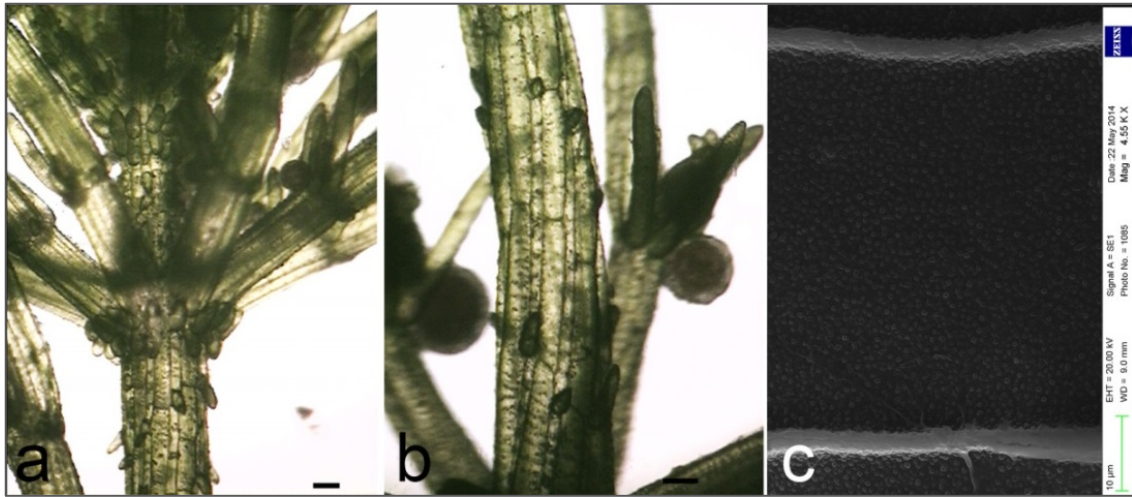


Figure 13. *Chara vulgaris* in Pool 4, March 2012: a - axis with stipulodes and base of whorl; b - axis with axial cortex and germination cells; c - oospore wall structure, SEM. Scale bars: a, b - 100 µm.



Figure 14. *Chara vulgaris* var. *longibracteata* (Kütz.) J. Gr. et B. Webst. from Dr. Lipkin collection: a - axis with stipulodes and base of whorl, antheridia pointed by white arrowhead; b - oospore pointed by white arrowhead; c - branches end cells; d - view of thallus. Scale bars: a - 200 µm; b - 50 µm; c - 50 µm; d - 1 mm

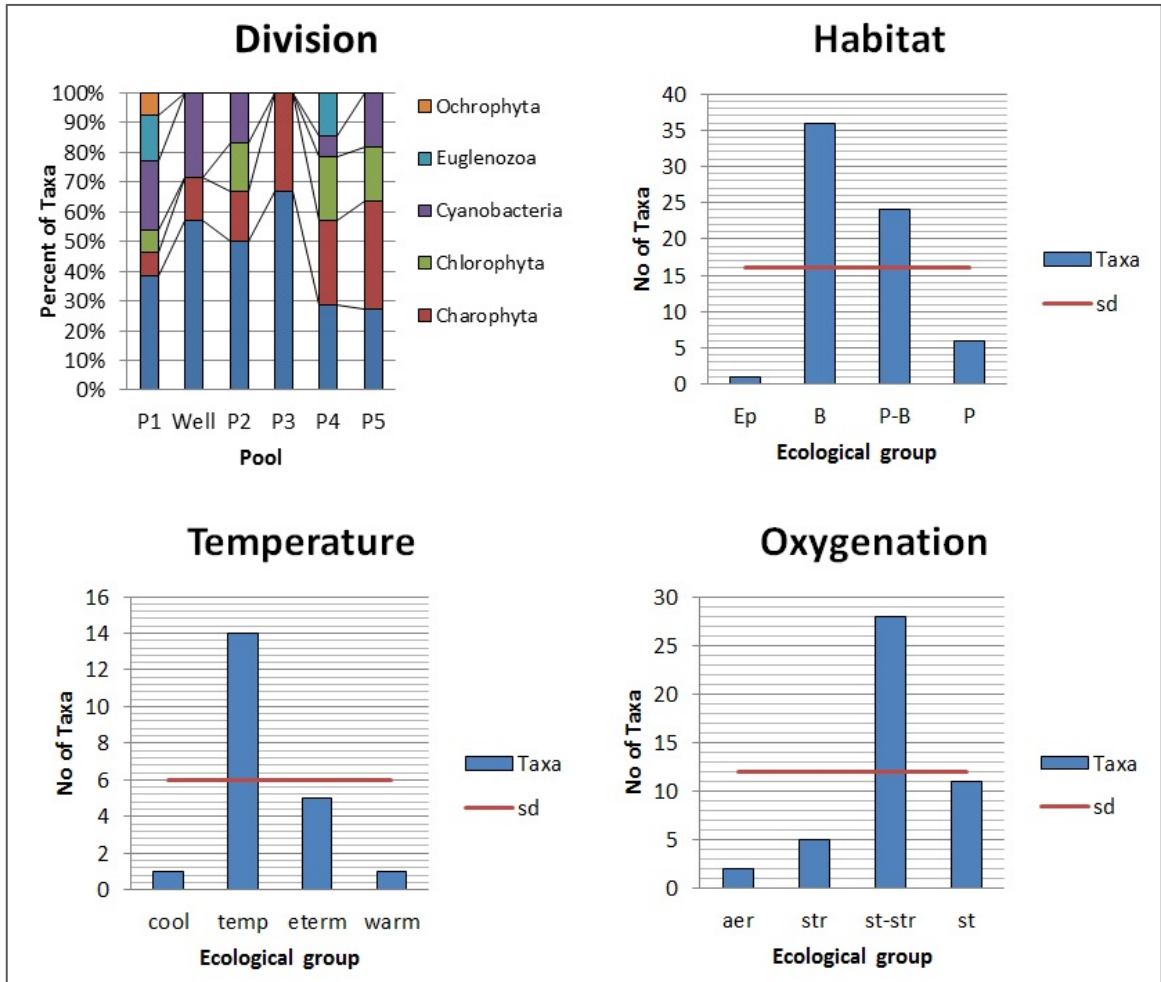


Figure 15. Taxonomic composition of algal communities and bio-indication in the pools of the Ein Afeq Natural Reserve

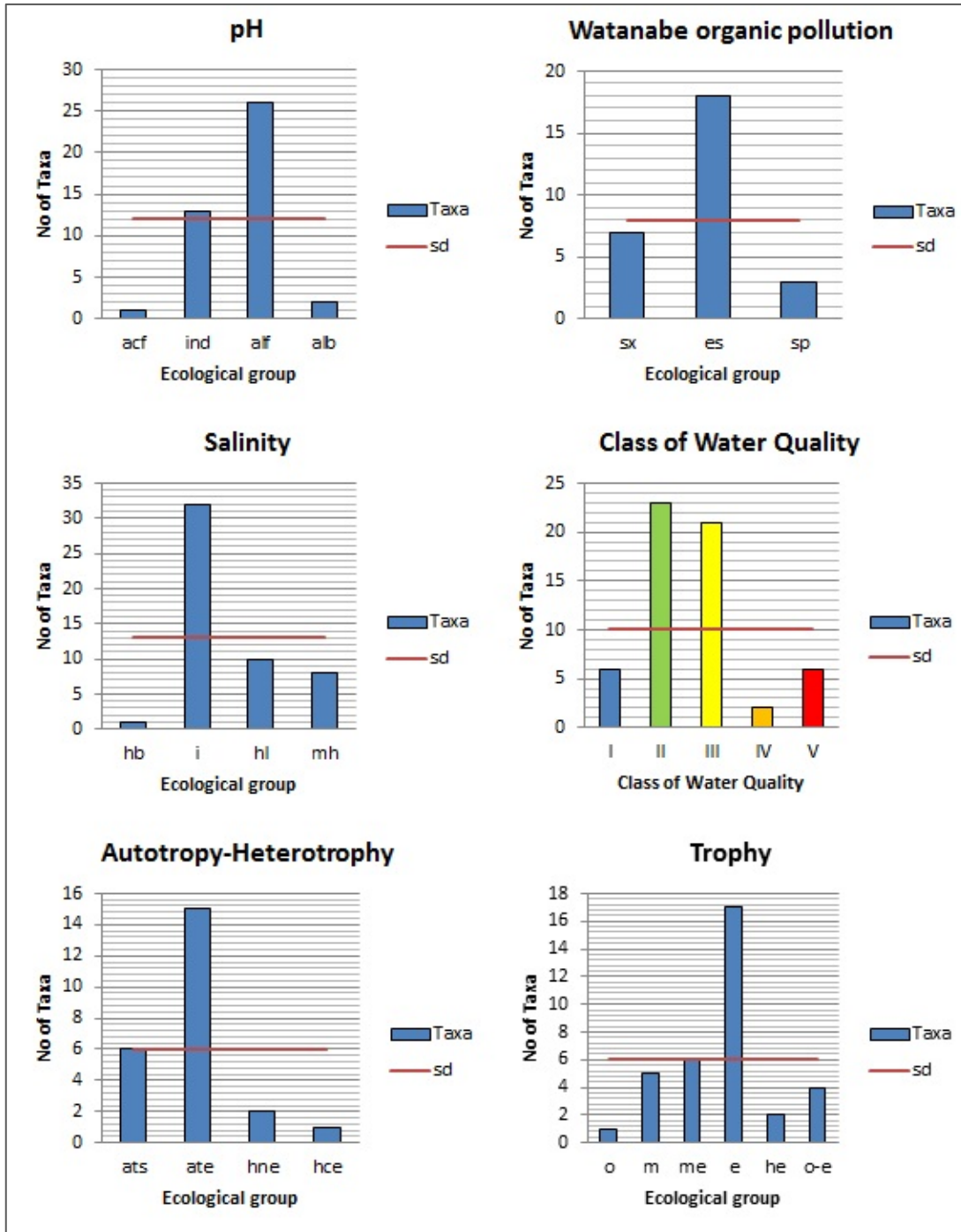


Figure 16. Bio-indication in the pools of the Ein Afeq Natural Reserve, where column in the plot of Class of Water Quality colored in European Standards

3.3. Bio-indication of the Studied Pool Environment

We use bio-indication methods in purpose to characterize of the studied pools water quality and ecosystem sustainable. As can be seen in Table 2, the water quality defined by bio-indication is the same that show by water chemistry (Table 1). In addition we can characterize the Ein Afeq Natural Reserve pools as low alkaline, fresh, eutrophic (Figure 16) with prevailing of organisms with autotrophic type of nutrition, which are mostly attached of substrate

(Figure 15) in moderate temperature medium oxygenated waters.

We calculated ecosystem state index WESI on the base of index Saprobity and nitrate-nitrogen concentration (Table 1). Can be seen that WESI is 0.40-0.80 in pools 2, 3 and 5, that mean that its ecosystems are under some press may be as a result of the organic enrichments come from the catchment area as a result of recreation. Present of the anoxia indicator species cyanoprockaryote *Pseudanabaena redeckei* (Table 2) confirm that Pool 2, deepest, is under

organic pollution impact. Pools 1, 4 and Well ecosystems are in good condition during study period. This situation is similar to that of the Upper Jordan River previously examined by us [18] where the pollution coming from the catchment area pollutes the water.

4. Conclusions

The Ein Afeq Natural Reserve pools as new studied locality in protected area of the Akko Plain in the Northern Israel can be characterize as natural, fresh, low alkaline with low-to-middle organic polluted waters that inhabit by seventy one algal species from which the streptophytes *Chara vulgaris* and *C. connivens* (Characeae) were found in well condition with attached mostly by diatoms. The both charophyte species are distributed over the Mediterranean phytogeographic realm and therefore can be used as distinct indicator of successional stage of aquatic ecosystem. The Ein Afeq pools ecosystem found as slightly impacted in few of them in a result of recreation. Therefore, the new charophyte locality in protected area of the Northern Israel can be monitored with using of chemical and bio-indication methods. It needs more attention to the pools management in purpose to protect from excessive recreation.

Acknowledgements

We thank Prof Yaakov Lipkin for collection of past samples, Dr Marcelo Sternberg for the possibility to study of herbarium specimens (TELA), Dr Sofa Marman, Svyatoslav, Nataly, Alex and George Rybnikov for their assistance in the field trip. This work was partly funded by the Israeli Ministry of Absorption and Israeli Taxonomic Initiative Fond, and Russian Foundation for Basic Research, project 14-04-31596-mol-a.

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